EFFECT OF TEMPERATURE AND SALINITY ON THE OXYGEN CONSUMPTION OF TWO INTERTIDAL CRABS [1]

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Studies on the effect of temperature change in poikilotherms have demonstrated physiological adaptations which permit varying degrees of independence of environmental temperatures. Various rate functions proceed within certain temperature limits, at activity levels which show compensatory responses to temperature. Mechanisms that result in such adaptations allow for degrees of thermal homeostasis in physiological processes. Latitudinally, thermal acclimation of rate functions has been demonstrated for many marine poikilotherms (Rao, 1953; Scholander *et al.,* 1953; Dehnel, 1955, 1956; Roberts, 1957b; and many others). Seasonal acclimation to temperature is documented (Edwards and Irving, 1943a; Clark, 1955; Roberts, 1957b), as well as intertidal vertical distribution (Segal, 1956). A general conclusion, based on intra- and interspecific comparisons, may be drawn from these data. Rate functions, such as weight-specific oxygen consumption, growth, heart beat, show that northern latitude populations, at their own natural temperature, have activity rates comparable to winter-adapted populations (at other latitudes), and low intertidal populations. Further, these three groups, when compared with their ecological opposites (southern latitude, summer-adapted, or high intertidal populations), at the same experimental temperature have generally higher rates of activity. Bullock (1955) and Prosser (1955) have documented extensively these considerations for poikilotherms.

Another environmental parameter, which has been given considerable attention by physiological ecologists is the effect of salinity on animal activity. Some of these studies have concerned mainly responses of poikilotherms to various osmotic concentrations, and determinations of gain and loss of water and ions in body fluid and urine, and accompanying weight changes (Jones, 1941; Robertson, 1949, 1953; Gross, 1954, 1955, 1957; Prosser, Green and Chow, 1955).

Other studies have concerned the effect of various osmotic conditions on metabolic activity. Flemister and Flemister (1951) working with *Ocypode albicans* determined that oxygen consumption at 26° C. was lowest in sea water (378 mM. Cl/L.), which was isotonic with crab blood, this water being considerably more hypotonic than field conditions at the time of collection (480 mM. Cl/L.). As the concentration of sea water varied from isotonicity, oxygen consumption increased, the highest rates being found in hypotonic solutions. Lofts (1956) compared respiratory rates at various salinities from tap water to 65‰, of two populations of the prawn *Palaemonetes varians,* one from a low salinity environ-

ment (1.3‰ NaCl) the other from a high salinity one (23.5‰ NaCl). In both groups the rate of oxygen consumption decreased as the salinity increased from tap water. Minimal respiratory rate for the high salinity population was at a salinity of 26‰, a condition isotonic with the animals. Minimal rate for the low salinity population occurred at 6‰. This latter value is somewhat hypertonic to the environment, and the prawns from this area are correspondingly hypertonic. Oxygen consumption increased as the experimental salinities increased beyond those of the natural environment. Gross (1955) has shown oxygen consumption to decrease as a function of desiccation in *Pachygrapsus crassipes*. Short term desiccation (several hours) is believed not to be deleterious. In a later paper, Gross (1957) measured oxygen consumption in *Uca* at 16° C. as a function of external concentration of the medium. His results showed that oxygen consumption did not always increase with osmotic stress. These results disagree with those of Flemister and Flemister (1951) for *Ocypode*. Marshall, Nicholls and Orr (1935) found that oxygen consumption of *Calanus* measured in 50% sea water and 15° C. was about 70% of respiration recorded in normal sea water (34‰). No generalization may be given regarding the effect sea water concentration has on oxygen consumption.

More recently, the combined effects of temperature and salinity on animal activity have been investigated. Broekema (1941) used different temperature-salinity combinations to determine the combined effect of these parameters on length of life of the shrimp *Crangon crangon*. She found that the optimal salinity for two-year-old shrimp was about 33‰ at a temperature of 4° C., whereas at 20° to 22° C. the optimal salinity was 28 to 29‰. When temperature decreases, salinity must rise. This condition was noted also for younger shrimp, larvae and eggs. Isotonicity is partially dependent upon temperature; at 20° C. isotonicity occurs at about 21.5‰, at 4° C., at 23‰. Further, with respect to osmotic behaviour, as temperature drops blood concentration rises in the hypotonic portion of the salinity range, and falls in the hypertonic part. These data support the fact that *Crangon* tolerates low salinities better when the temperature is high. Smith (1955a) has suggested that summer salinities in the Baltic Sea are not limiting factors for *Nereis diversicolor*, but low spring salinities and temperatures adversely affect osmoregulatory abilities, thus presenting an ecological limitation. In another paper Smith (1955b) compared chloride regulation in several geographically separated populations of *Nereis diversicolor*. One population was collected from upper River Tamar, England, and the level of chloride regulation was determined at a series of temperatures (7°, 14° and 21° C.) and over a range of chlorinities (0.02 grams chloride/liter to 14.08 grams chloride/liter). For a given chloride content of the medium the three temperatures produced no significant differences in level of chloride regulation. Further work by Smith (1957) on chloride regulation, as affected by temperature, in populations of *Nereis lighti* from the Salinas River, California has shown that at chlorinities above 1.0 grams/liter, low temperatures (0.5° C.) did not affect chloride regulation, when adapted to low salinities prior to exposure to low temperatures. However, animals adapted to 0.5° C. initially, in a chlorinity of 2.0 grams/liter, and then transferred to fresh water, failed to show chloride regulation, but did show volume regulation. At higher adaptation temperatures (12° C.) salinity reduction did not result in lowering of the coelomic chloride level.

In the last few years Kinne (1953, 1956a, 1956b) in a series of papers has reported the physiological effect of temperature and salinity on several species of invertebrates, with particular reference to growth in hydroids. Kinne (1957) states (p. 90): "temperature can change (enlarge, narrow or shift) the salinity range, and salinity can change the temperature range of a species. The effect of a given temperature depends on the salinity and vice versa." Kinne and Rotthauwe (1952) have presented data for *Rithropanopeus harrisi* showing that as temperature decreases blood concentration rises along the entire length of the curve, except for salinities above approximately 30‰. This crab is hypertonic and normally lives in waters of very low salinity (1 to 5‰). *Rithropanopeus* has been shown to withstand low salinities better when the temperature is low. It should be pointed out that these conditions are the reverse to those reported by Broekema (1941) for *Crangon*. Kinne (1956b) observed growth and reproduction in the hydroid *Cordylophora caspia* under different temperature and salinity combinations. It was determined that these hydroids withstood high temperatures at high salinities better than at lower salinities.

The combined effect of temperature and salinity has been studied with regard to its influence on temperature tolerance. The pertinent literature has been reviewed recently (Todd and Dehnel, 1960). In this laboratory Todd and Dehnel (1960) investigated the influence of seasonal change and laboratory acclimation to various temperature-salinity combinations on heat tolerance of *Hemigrapsus oregonensis* and *H. nudus*. They found that there was a seasonal change in both species when winter and summer data were compared. Further, acclimation to a high temperature increased resistance to lethal temperatures and acclimation to low salinities decreased this resistance. For both seasons, winter and summer, a combination of high temperature and high salinity proved most favorable for resistance to lethal temperatures.

The present investigation is a study of the combined effect of temperature and salinity on respiratory metabolism in two species of intertidal crabs, *Hemigrapsus oregonensis* and *H. nudus*. The facts that both species, in this geographic locality, occupy similar ecological niches, occur in abundant numbers, and are maintained readily under laboratory conditions permit inter- as well as intraspecific comparisons. It has been demonstrated that the degree (absolute and relative) to which these crabs acclimate to a given parameter, either temperature and/or salinity, depends upon the season of the year at which they were collected and the experimental temperature-salinity combination imposed at either season. This work extended from the winter, 1955, to the summer, 1957.

MATERIAL AND METHODS

Abundance and suitability to laboratory conditions of these two eurytopic species, *Hemigrapsus oregonensis* (Dana) and *H. nudus* (Dana) permit studies of temperature and salinity acclimation. Schmitt (1921) lists distribution of *H. nudus* from Sitka, Alaska to the Gulf of California, and *H. oregonensis* from Prince William Sound, Alaska to the Gulf of California.

Collections of crabs were obtained from Spanish Bank (Latitude, 49° 17′ N; longitude, 123° 07′ W), Vancouver, British Columbia (Fig. 1). Specific areas on the beach were marked and animals were collected only from these regions. *H.*

oregonensis was collected at approximately the 7.0-foot tide level and *H. nudus* at the 9.0-foot tide level (based on Pacific Coast Tide and Current Tables, Canadian Hydrographic Service, Department of Mines and Technical Surveys). Sea water temperatures and samples were taken at each time of collection and salinity determinations were made on these samples. Animals were returned to the laboratory in canvas buckets containing dampened sea weed.

Habitat

The Spanish Bank area, from which populations of *H. oregonensis* and *H. nudus* are studied, borders the south shore of a relatively protected bay, which extends in an east-west direction. Extension of this beach forms a point (Point Grey), along the south side of which the Fraser River flows (Fig. 1). Spanish Bank beach is a rocky mud-sand intertidal area. The habitat for these crabs is the narrow restricted upper rocky area, ranging approximately from 3.0-foot tide level to 10.0-foot tide level. The linear distance is about 150 feet. The fauna of this area is relatively poor, major elements being *Mytilus edulis, Balanus glandula* and the two grapsoid crabs. These species abound in numbers. Below this, the beach extends its gradual slope into a mud-sand flat which continues for several hundred yards, before dropping to form the channel. This mud flat is exposed on low tides, and a similar paucity of fauna is due to extremely low summer salinities, a condition to be discussed later.

Latitudinal distribution of *H. oregonensis* and *H. nudus* is very similar. Ecologically, however, these two species are quite different. *Hemigrapsus nudus* is an open coast intertidal species, whereas *H. oregonensis* is an intertidal bay and estuarine species. However, in this geographic area, both species are found abundantly, occupying essentially the same habitat. Vertically, *H. oregonensis* is located lower in the intertidal, and ranges from approximately the 3.0-foot tide level (lower area of rocks) to the 8.0-foot tide level. *H. nudus* is higher intertidally, ranging from approximately 6.0-foot tide level to 10.0-foot tide level. The zone of *H. oregonensis* is defined much more clearly than that for *H. nudus*. The zones as given above suggest nearly comparable width, a condition which existed at the beginning of the work, 1955–1956. For the past two years, there has been a relative stability of the *H. oregonensis* zone, but the zone of *H. nudus* has shifted progressively to a lower position, intertidally. The zone of overlap is broadening and the lower level of *H. nudus* now is approximately at the 4.0-foot tide level. At present, due to apparent contour changes of the beach, definition of areas is much less evident. Individuals of both species characteristically are found under the same rock, particularly in the area of overlap, a condition which changes somewhat seasonally. The usual niche occupied by *H. oregonensis* is under rocks, frequently partially or completely buried in the mud, which contains considerable amounts of decaying organic matter. This species is found also in beds of *Mytilus edulis*. *H. nudus* is found under rocks, infrequently in mud, and also in *Mytilus* beds, but generally occupies a much less sedimented microhabitat.

During the winter and early spring the zone of overlap is reduced and the areas occupied by the two species are defined more clearly. This zonation appears to be correlated with breeding activity. The breeding season for *H. nudus* is from January to May, and for *H. oregonensis*, from February to June. At the beginning
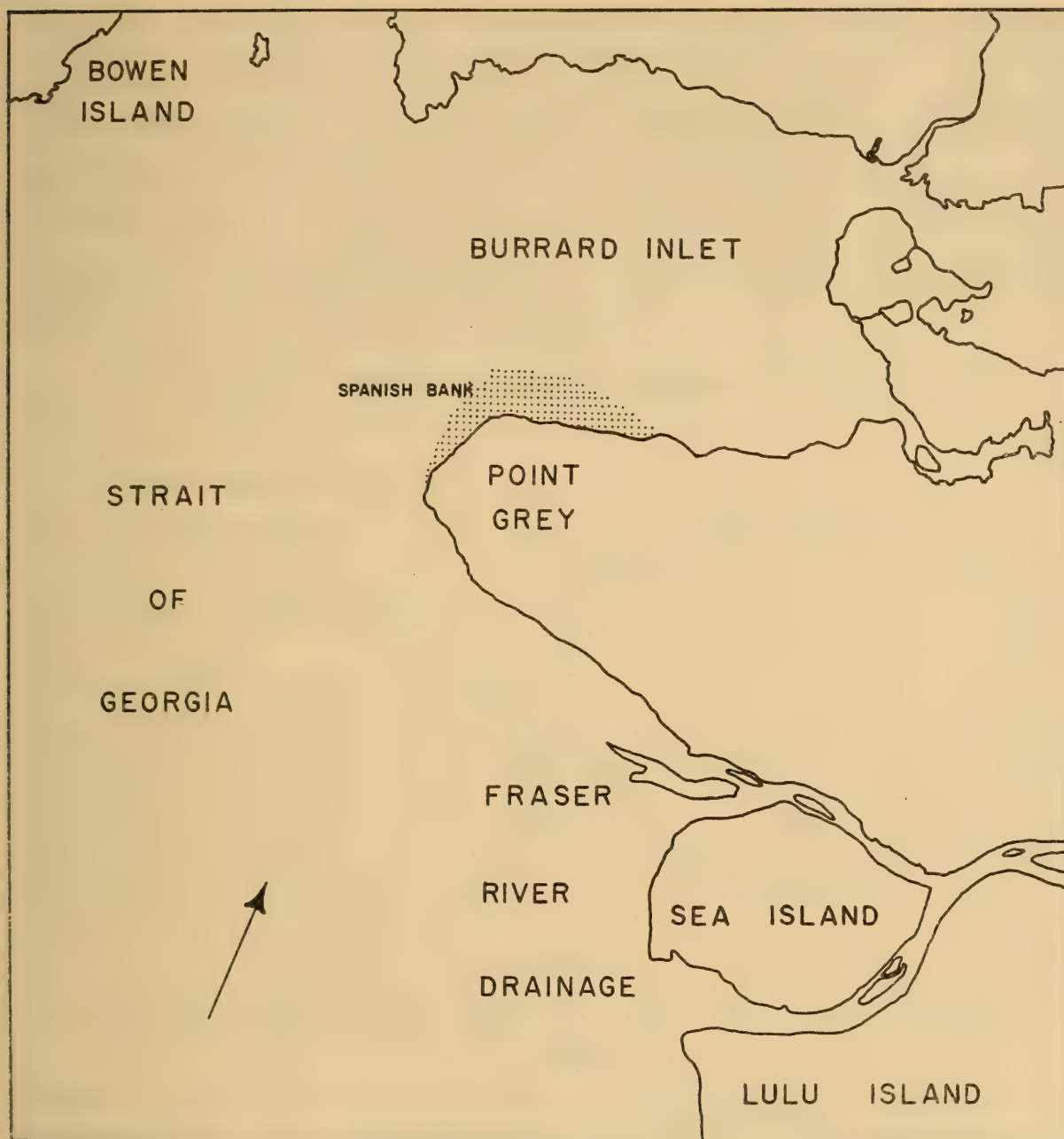
FIGURE 1. Map of the Spanish Bank area where this study was conducted. Note proximity of the Fraser River drainage which is responsible for seasonal fluctuations in salinity.

of the breeding season the two species separate spatially. After the females lay eggs, the zone of overlap is increased and the two species intermingle. Evidence is available (to be published elsewhere) which demonstrates a degree of interbreeding between these two species. An intergrade series includes crabs ranging from *H. oregonensis* with characters of *H. nudus* to the opposite extreme. In other regions of their distribution, ecological conditions such as low salinity seem to serve as barriers and prevent interbreeding. In this area, however, no such barrier apparently exists.

Temperature-salinity conditions in this area are rather severe, and warrant a

description. These relations undoubtedly serve to limit, ecologically, invasion by a more varied fauna. Seasonal variations impose strict limitations with regard to temperature and salinity tolerance, and only a select group appears to have adequate regulatory mechanisms to compensate for these changes. In adjacent regions where the Fraser River does not have an effect, the fauna is more plentiful and varied.

Previously, it was stated that this beach area extends westward and the south-facing region borders the Fraser River. Volume of this river changes seasonally, flooding in the spring and summer, due to interior British Columbia runoff. This volume of water flows into the Strait of Georgia and currents carry the low saline, low density water mass into adjacent areas and around Point Grey into the collecting
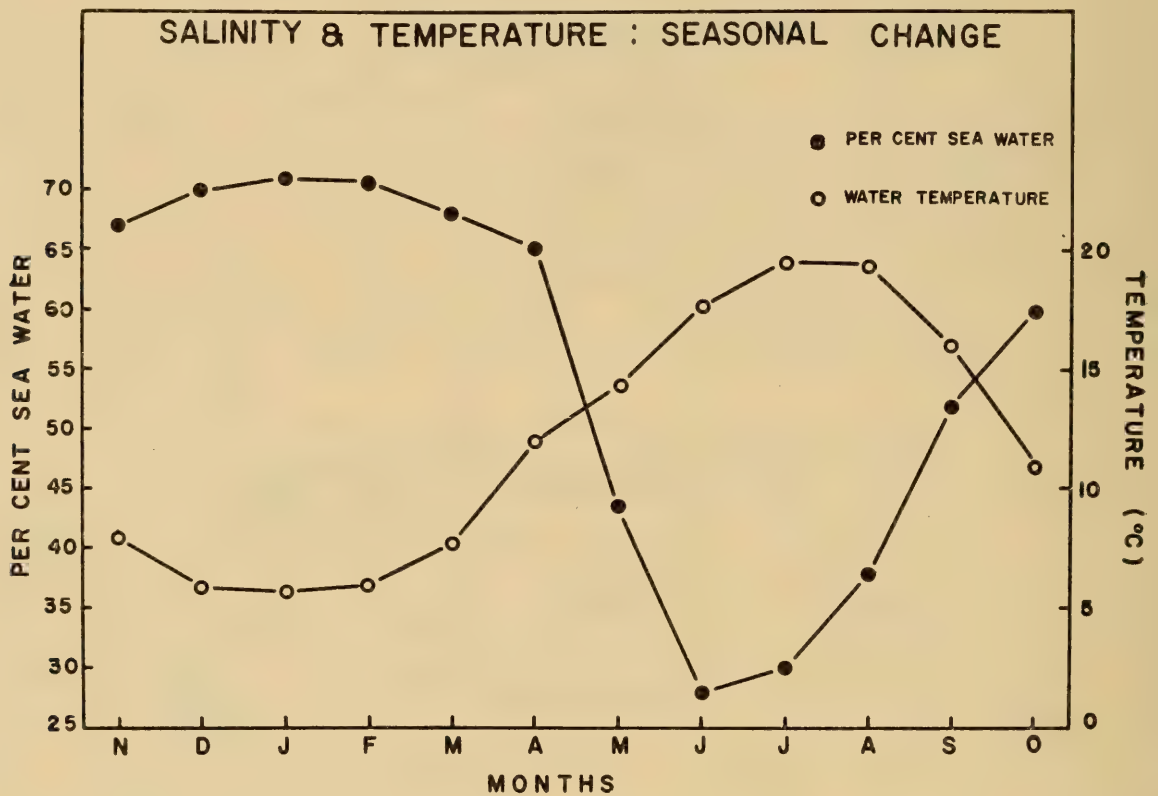

FIGURE 2. Mean monthly intertidal sea water temperatures and salinities for Spanish Bank, Vancouver, British Columbia (1955–1959). Solid circles (●) represent sea water salinity (%), open circles (○) represent sea water temperature (° C).

area. This fresh water influences greatly the intertidal salinity of Spanish Bank, not only seasonally but during a twenty-four-hour high-low tidal cycle.

During the winter, intertidal sea water temperatures range from 1.0° C. to 6° C. (Fig. 2). Isolated pools at low tide (which occur during the late evening and early morning) have been recorded as low as − 0.5° C. Fraser River runoff is at a minimum and local salinities vary from 70% to 80% sea water. These conditions exist from approximately the end of November until the end of February. Winter conditions with regard to these two parameters are relatively stable ones. Spring conditions are transient, involving a rise in temperature and a drop in salinity. Temperature in late February and early March begins to rise, prior to an appreciable salinity change, from approximately 5° to 8–10° C. Following this,

salinity begins to drop to about 50% sea water. Spring conditions merge into summer conditions, which extend from approximately the end of May to the end of August. Spring temperatures continue to rise and salinities drop until average summer intertidal temperatures of approximately 20° C. and summer salinities of 25% to 35% sea water are obtained. Again, for several months relatively stable temperature-salinity conditions exist. During the fall, from approximately August to November, a transient temperature-salinity period again exists. Fall salinities rise relatively more rapidly and earlier than the seasonal lowering of temperature. Stability, low temperature, high salinity, is reached during December and the seasonal temperature-salinity cycle is completed.

In this geographical region the usual two low, two high tides per twenty-four hours are interrupted frequently and only one low or one high tide results. This condition is important conceivably during the summer months. Off shore, a line of demarcation results between low salinity, low density Fraser River water with higher salinity, higher density sea water. During a high tide, sea water over the intertidal region is mixed well, and the usual low summer salinity exists. As the tide drops, salinity remains low until the incoming tide brings in initially lower salinity water, and mixing occurs. This tidal fluctuation in salinity might be of the magnitude of 15% to 45% sea water.

Seasonal rate-temperature experiments

Male crabs of both species, ranging from approximately 0.3 grams to 6.0 grams, were collected for these experiments and were placed in plastic containers (10½″ × 13″ × 4½″) with lids, in approximately 3.5 liters of sea water. Each container held approximately 30 animals of one species. Lids were left ajar, and a gauze cloth was placed in the container to help separate the animals. Sea water salinity approximated field salinity at the time of collection. Containers were placed in temperature-controlled refrigerators ($\pm 1.0°$ C.) for approximately twenty-four hours, a sufficient time to allow partial clearing of the gut. Holding temperature approximated field conditions at the time of collection. Crabs were kept in darkness and not fed. Following the twenty-four-hour holding period, oxygen consumption was measured at a series of temperatures, 2°, (winter), 3.5°, (summer) 5°, 10°, 15°, 20°, 25°, 27°, and 30° C. In some cases oxygen consumption at two different temperatures was measured in one day. All experiments were commenced at approximately the same time each day. The following day another group was measured, these animals having been held under constant conditions of temperature and salinity for a maximum time of forty-eight hours. New collections were made to complete the series. For each experiment, measurements were recorded for twenty-four crabs. Crabs were discarded after measurements were completed for one day. Animals were changed daily with water of the appropriate salinity and temperature.

Respiration studies on individuals respiring in air can be carried out most conveniently on *H. oregonensis* and *H. nudus* because of the intertidal environment occupied. In all respiration studies measurements were made with the Wennesland modification (1951) of the Scholander microrespirometer (1949). Crabs were placed in darkened chambers with sufficient sea water to keep the gills moist, and then transferred to a constant temperature water bath ($\pm 0.1°$ C.). Equilibration

time varied with the change between holding temperature and experimental water bath temperature. One hour for thermal equilibration was always allowed, with the respirometers open to the atmosphere. A further forty-five minutes to one hour was allowed for each additional 5° C. change in temperature. Following thermal equilibration, the respirometers were closed and successive readings were made at ten-minute intervals. Each experiment lasted from one and one-half hours to two hours, based on the time required for respiration levels to become linear with time. Following the experimental period crabs were removed from the respirometer chambers, dried with gauze and weighed to the nearest 0.1 gram. Data on individuals were discarded if the animals showed activity, or if high or low respiratory rates were obtained, due to approaching death. Respiration rates were given as cubic millimeters of oxygen consumed per gram per hour (weight-specific oxygen consumption). Data for each experimental temperature were plotted as weight-specific oxygen consumption as a function of weight on log-log paper. Regression lines were fitted by the method of least squares. Respiratory rate for a given weight animal was plotted against temperature on semi-log paper to demonstrate the rate-temperature relationship.

Temperature and salinity experiments

Methods of collecting and holding were similar to those described for rate-temperature experiments. For the temperature acclimation experiments, four temperatures were chosen, 5°, 10°, 15° and 20° C. (± 1.0° C.). Animals were acclimated for at least one week, maintained under dark conditions and not fed. Experiments were arranged in such a fashion that a complete series of measurements was obtained on one species prior to commencing the other. Experiments were conducted during the late spring and summer months. In one series of experiments, at four acclimation temperatures, a constant salinity of 75% sea water was used, which corresponded to winter field conditions. In another series, a constant salinity of 25% sea water was used, which corresponded to summer field conditions. At the end of one, two and in some cases three weeks, oxygen consumption measurements were made on groups of crabs acclimated to each of the four temperatures and a constant salinity. Experimental water bath temperatures used were 10° and 20° C. (± 0.1° C.). Respiratory measurements were determined as mentioned previously. After the 10° C. experimental temperature was completed, the water bath was raised to 20° C. (time required approximately one hour) and the animals were allowed one and one-half hours thermal equilibration. Following completion of the experiment and weighing, animals were returned to the experimental temperature and salinity conditions. Results were not different when animals were measured initially at 20° C. and then lowered to 10° C. Respiration rates were given as cubic millimeters of oxygen consumed per gram per hour (weight-specific oxygen consumption). Data for each acclimation temperature-salinity combination at each experimental temperature were plotted as weight-specific oxygen consumption as a function of weight on log-log paper. Regression lines were fitted by the method of least squares.

All field and experimental salinities are expressed as percentage sea water, based on a standard sea water, 31.88‰ salinity, 17.65‰ chlorinity at 25° C., as 100% sea water. Sea water concentrations were prepared from local sea water, away from the influence of Fraser River runoff, which ranged from approximately

90% sea water in the winter to 65% in the summer. For concentrations below normal sea water, dechlorinated fresh water was added until the desired dilution was obtained. For concentrations higher than normal sea water, sea salt was added, based on 31.88 grams per liter of sodium chloride. Experimental salinities were determined on a 1000-cycle conductivity bridge calibrated to the standard sea water noted above.

METHOD OF ANALYSIS

As stated previously rate of oxygen consumption was plotted as a function of body weight (weight-specific) on a double logarithmic system. Such a plot, over an adequate weight range, gives a straight line with a negative slope. Regression of weight-specific oxygen consumption against body weight assumes the form:

$$\frac{O_2}{W} = aW^{b-1}$$

or:

$$\log O_2 - \log W = \log a + b \log W - \log W$$

where O_2 is oxygen consumption of the crab in mm.[3] O_2/gram/hour, W is body weight in grams, a the intercept and b the slope of the line. Negative linear regression coefficients were calculated by the method of least squares. It was necessary to determine whether statistically significant differences existed between regression lines representing metabolic rates of crabs measured at different combinations of acclimation temperatures (5°, 10°, 15° and 20° C.), and salinities (25% and 75% sea water) at the two experimental temperatures (10° and 20° C.). Each calculated regression line was based on oxygen consumption measurements of at least thirty-five crabs and in most cases fifty to sixty. These data are suited to statistical treatment by analysis of covariance, a standardized method outlined by Ostle (1954) for a randomized sample. The method was to test by analysis of covariance the null hypothesis that no true differences existed in the effect of different combinations of temperature and salinity on oxygen consumption, *i.e.*, that two regression lines could be represented by a single regression line. In the analysis, negative logarithm values were eliminated by multiplying oxygen consumption rates by 10 before converting to four place common logarithms.

Zeuthen (1953) has suggested that the terms "respiratory rate," "metabolic rate" and "rate of oxygen uptake" be defined as "oxygen uptake per hour per unit of body size." This procedure was observed here. Further, "weight-specific oxygen consumption" connotes the same meaning. Use of the word "acclimation" is preferred to "acclimatization" following the suggestion of Bullock (1955; see also Prosser, 1955). This word refers to intra- and interspecific compensatory changes whether these changes be phenotypic or genotypic. Other descriptive words such as "regulation," "compensation" and "homeostasis" are used with no other implications than stated above.

RESULTS

Seasonal rate-temperature experiments

Results of winter and summer oxygen consumption at various experimental temperatures, for crabs removed directly from field conditions, are shown in Figure 3 (*H. oregonensis*) and Figure 4 (*H. nudus*). Crabs of both species with a weight of 2.0 grams have been chosen arbitrarily to depict these data.

Hemigrapsus oregonensis: Respiratory metabolism for *H. oregonensis* consistently is higher for summer animals than for winter ones. These data demonstrate the fact that there is no acclimation of oxygen consumption to temperature by this species under field conditions. Shape of the winter and summer curves is similar in the physiological temperature range. Winter animals show no cold depression at 2° C. and some heat depression occurs between 25° and 27° C. Summer crabs are depressed greatly at 3.5° C., Q_{10} value between 3.5° and 5° C. is 836.0, and heat depression is above 27° C. Depression between 27° and 30° C. is somewhat less for winter crabs. Physiological temperature range for summer
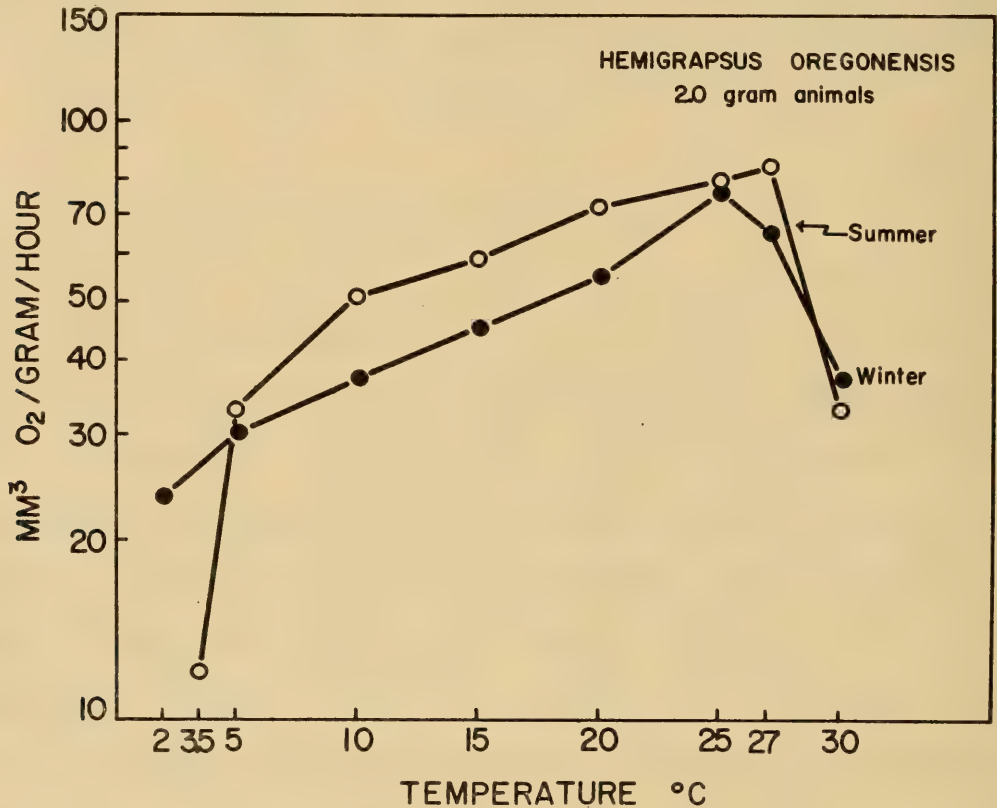

FIGURE 3. Seasonal rate-temperature curves, acutely measured, for 2.0-gram *Hemigrapsus oregonensis*. Respiratory rates for this weight crab were chosen from regression lines determined for weight-specific oxygen consumption data at each temperature. Lines were fitted by the method of least squares. Summer animals (○) were kept in 25% sea water and winter animals (●) in 75% sea water, prior to and during the experimental period.

crabs is about 5° C. to 27° C., whereas that range for winter crabs is 2° C. to 25°–27° C.

Hemigrapsus nudus: In Figure 4 approximately the same conditions as noted for *H. oregonensis* are presented for *H. nudus*. Summer-adapted *H. nudus* consistently are higher than winter ones, showing no respiratory acclimation to temperature under field conditions. Shape of the two curves is somewhat similar from 10° C. to 25° C. Consideration of the ends of the curves allows further discussion. Winter-adapted *H. nudus* show no cold depression at 2.0° C. whereas summer-adapted *H. nudus* show considerable depression below 5° C. The Q_{10} value between 3.5° and 5° C. is 166.5. At the higher end of the temperature

range, winter-adapted *H. nudus* show some heat depression between 25° and 27° C.
Summer-adapted *H. nudus* likewise are depressed between 25° and 27° C. De-
pression between 25° and 30° C. is considerably less for winter than for summer
animals. Physiological temperature range for summer crabs is about 5° to 25° C.,
whereas that range for winter crabs is 2° C. to 25°–27° C.

Interspecific comparison: Seasonal comparison shows that summer *H. ore-
gonensis* have a slightly higher absolute oxygen consumption below 15° C. for
any given weight and temperature, than does summer *H. nudus* for that same weight
and temperature; 20° C. and above, summer *H. nudus* have a higher absolute
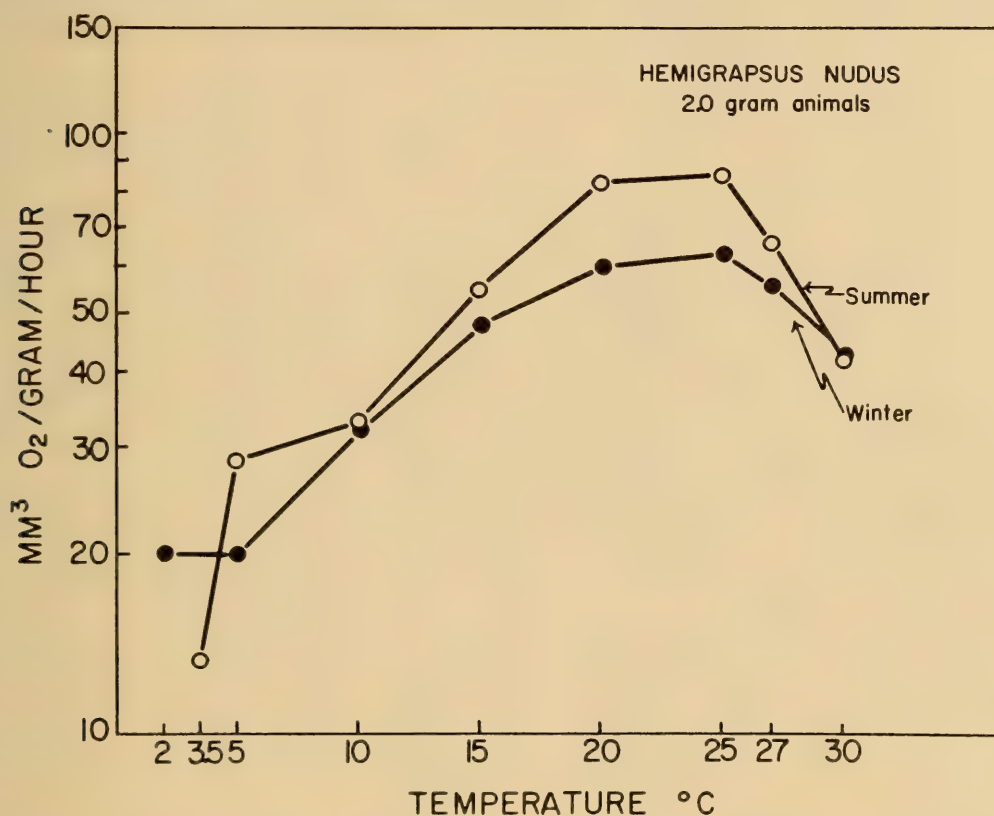oxygen consumption. Approximately the same rate is found at 15° C. Winter

FIGURE 4. Seasonal rate-temperature curves, acutely measured for 2.0-gram *Hemi-
grapsus nudus*. Respiratory rates for this weight crab were chosen from regression lines
determined for weight-specific oxygen consumption data at each temperature. Lines were
fitted by the method of least squares. Summer animals (○) were kept in 25% sea water
and winter animals (●) in 75% sea water, prior to and during the experimental period.

crabs of both species have about the same rate of metabolism at 15° and 20° C.
for any given weight. At other temperatures, the rates for *H. oregonensis* are
higher.

Both of these species fail to show a shift on the ordinate, winter versus summer
(an acclimation shift), but neither do they show any appreciable difference at the
high end of the temperature range. It would be expected that summer animals
conceivably would show depression of oxygen consumption at a higher temperature
than winter crabs. Actually, winter *H. nudus* extend their upper limits to about
the same level as summer ones. Summer *H. oregonensis* has a slightly higher

limit than do winter crabs. If the summer curves for both species are considered as baselines, then it can be argued that winter animals are showing an acclimation of the upper physiological limit. They do not show the drop to a lower temperature associated with cold water living. Correspondingly, winter animals have extended their curves to the left (lower temperature before depression) and winter animals have a somewhat wider physiological temperature range of oxygen consumption (2.0° C. to 25°–27° C., both species) than summer animals (5° C. to 27° C., *H. oregonensis*, 5°–25° C., *H. nudus*). Absolute temperature range for summer *H. nudus* is 20° C. and summer *H. oregonensis* is 22° C., whereas that same range for both species of winter crabs is about 23°–25° C. Such a condition is the reverse to that found by Segal (1956) for seasonal variations in heart beat of *Acmaea limatula,* and for growth rates of latitudinally displaced populations of gastropod larvae reported by Dehnel (1955).

Another method which was used for comparison between two curves is Q_{10}, provided limitations are recognized and accepted. Over physiological ranges of temperature, Q_{10} values express variations in temperature sensitivity of oxygen consumption under different thermal conditions, if intervals between temperatures at which Q_{10} values are determined are relatively small. The Q_{10} values determined for cold- or heat-depressed regions of the rate-temperature curves, for instance, would have no biological meaning unless the depression were shown to be fully reversible or to occur under normally encountered conditions. Evidence obtained from temperature tolerance experiments (Todd and Dehnel, 1960) shows that heat depression is reversible in both species, winter and summer, with respect to 50% mortality levels at much higher temperatures than reported here.

Comparison of Q_{10} values, winter versus summer, shows little consistency. Between 10° and 20° C. summer *H. nudus* have considerably higher Q_{10} values than winter ones (summer, 10°–15° C., $Q_{10} = 2.8$; winter, $Q_{10} = 2.3$; summer, 15°–20° C., $Q_{10} = 2.3$; winter, $Q_{10} = 1.6$). At other parts of the temperature range, winter crabs have higher values. Winter *H. oregonensis* between 10° and 25° C. have higher Q_{10} values (winter, 10°–15° C., $Q_{10} = 1.5$; summer, $Q_{10} = 1.3$; winter, 15°–20° C., $Q_{10} = 1.5$; summer , $Q_{10} = 1.5$; winter, 20°–25° C., $Q_{10} = 1.9$; summer, $Q_{10} = 1.2$). At other parts of the temperature ranges summer crabs have higher values.

Scrutiny of Q_{10} values for both species, winter and summer, shows many values to be much lower than the generally accepted value for poikilotherms, 2.0 to 3.0. Lower values are seen fairly consistently over physiological ranges of temperature, particularly for *H. oregonensis*, winter and summer.

Effect of acclimation temperature

Hemigrapsus oregonensis: When the four acclimation temperatures (5°, 10°, 15° and 20° C.) are compared at either low (25% sea water) or high (75% sea water) salinity at 10° C. experimental temperature, it is seen that as acclimation temperature increases oxygen consumption decreases over most of the weight range (Figs. 5 and 6). Analysis of covariance of the total of the four regression lines at either salinity, with respect to their position on the ordinate, gives a P value of less than one per cent (Table I). In the case of *H. oregonensis* (Fig. 5) when 1.0-gram crabs are compared at 5° C. and 20° C. and a salinity of 25%

sea water, there is an 87% increase at the lower acclimation temperature. The difference between these two regression lines is statistically significant (P = 0.01). Comparisons of the 5° C. acclimation temperature with the two intermediate temperatures similarly show significant differences at the one per cent level of

TABLE I

Analysis of covariance of rates of oxygen consumption per gram per hour as a function of weight in Hemigrapsus oregonensis *and* H. nudus *at 10° C. experimental temperature, at two salinities (25% and 75% sea water) and four acclimation temperatures (5°, 10°, 15° and 20° C.). Combinations of acclimation temperatures are compared at each salinity. P indicates the significance of the position of the regression lines on the ordinate. P_b indicates the significance of the change in slope of the regression lines. b is the regression coefficient. r indicates coefficient of correlation*

Accl. sal. (%)	Accl. temp. (°C.) comparisons	P	P_b	Accl. temp. (°C.) b	r
	Total	0.01	0.01	5° C. −.678	−.8641
	5 and 10	0.01	N.S.		
	5 and 15	0.01	N.S.	10 −.685	−.8205
25	5 and 20	0.01	0.01		
	10 and 15	N.S.	N.S.	15 −.666	−.8322
	10 and 20	0.05	0.01		
	15 and 20	N.S.	0.01	20 −.411	−.8081
H. oregonensis					
	Total	0.01	N.S.	5° C. −.589	−.9345
	5 and 10	0.01	N.S.		
	5 and 15	0.01	0.01	10 −.591	−.8216
75	5 and 20	0.01	N.S.		
	10 and 15	N.S.	0.05	15 −.361	−.8338
	10 and 20	N.S.	N.S.		
	15 and 20	N.S.	N.S.	20 −.333	−.5208
	Total	0.01	N.S.	5° C. −.511	−.8296
	5 and 10	0.01	N.S.		
	5 and 15	0.01	N.S.	10 −.517	−.8525
25	5 and 20	0.01	N.S.		
	10 and 15	N.S.	N.S.	15 −.578	−.8793
	10 and 20	0.01	N.S.		
	15 and 20	0.01	N.S.	20 −.520	−.9109
H. nudus					
	Total	0.01	0.05	5° C. −.500	−.7598
	5 and 10	N.S.	N.S.		
	5 and 15	0.05	N.S.	10 −.441	−.8518
75	5 and 20	0.01	0.05		
	10 and 15	0.05	N.S.	15 −.459	−.7022
	10 and 20	0.01	0.01		
	15 and 20	0.01	0.05	20 −.651	−.9419

probability. Further, statistical comparisons of acclimation temperatures in various combinations are given in Table I.

At the lower salinity (Fig. 5) it is seen that the regression lines, with the exception of the 5° C. acclimation temperature, converge toward the higher end of the weight range, at approximately 2.0 to 3.0 grams. Analysis of the four lines shows a statistically significant difference (P = 0.01) in change of slope. If the
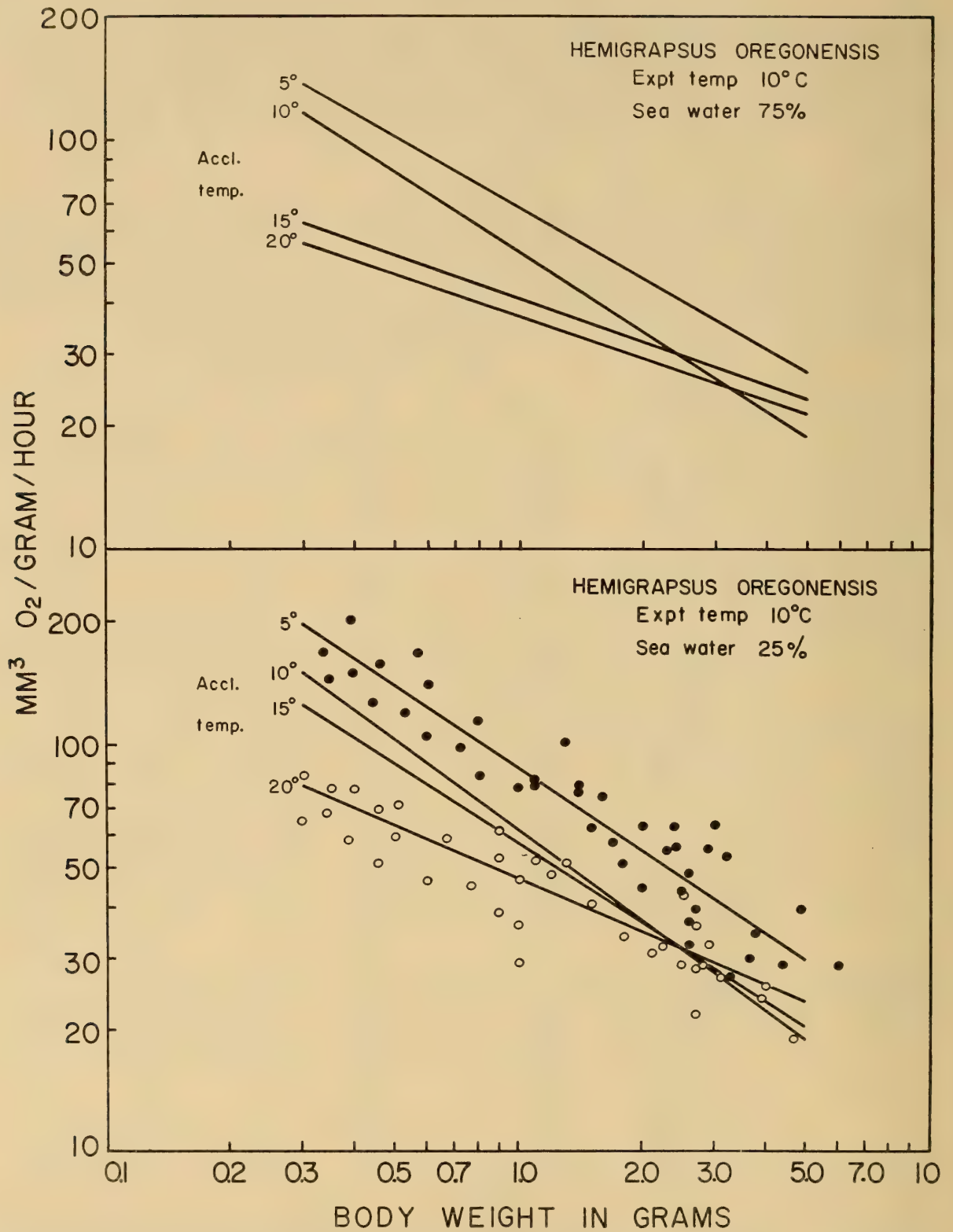
FIGURE 5. Effect of acclimation temperature on weight-specific oxygen consumption with increasing size in *Hemigrapsus oregonensis,* at the two acclimation salinities, 25% sea water (lower) and 75% sea water (upper). Each point represents an animal. Points for 5° C. (●) and 20° C. (○) are included to demonstrate variation. Regression lines were fitted by the method of least squares. Slope values, coefficients of correlation and other statistical data are given in Table I.

four acclimation temperatures are compared, as these temperatures increase the slopes of the regression lines are nearly parallel, whereas the 20° C. line is significantly different (P = 0.01) from the other three (Table I). If the rate of oxygen consumption of a 2.5-gram crab is compared at 5° C. and 20° C., the increase is 47% at the lower temperature. This same difference exists for the two intermediate acclimation temperatures when either is compared with the 5° C. regression line. This increase is approximately one-half that determined for a 1.0-gram animal at the low and high acclimation temperatures.

At the higher salinity, 75% sea water, *H. oregonensis* shows a similar trend to that discussed for the lower salinity (Fig. 5). Comparison of 1.0-gram animals at low and high acclimation temperatures shows an 83% increase in weight-specific oxygen consumption at the lower temperature. Further comparison of the 5° C. line with the two intermediate acclimation temperatures results in levels of probability of the same magnitude but less percentage differences (Table I). Combinations of intermediate acclimation temperatures within themselves or with high and low temperatures result in the same degrees of significance or insignificance as noted for the response to low salinity in this species.

Again as with the low salinity, slopes (*b* values) of the four acclimation temperatures decrease as those temperatures increase. Comparing the change of slope of the four acclimation temperature regression lines, analysis shows that there is no significant difference (Table I). Specifically, regression lines of the two lower temperatures are nearly parallel, as is the case for the two higher acclimation temperatures, and no significant differences are found to exist within these two pairs. However, comparison of only the 5° C. line with the 15° C. one gives a statistically significant difference (P = 0.01). There is a tendency for the 10°, 15° and 20° C. acclimation temperature lines to converge at the 2.0- to 3.0-gram weight. This is the same pattern as found at the lower salinity. Again, if a 2.5-gram crab is compared at 5° C. and 20° C. acclimation temperatures, there is found a 48% increase in oxygen consumption at the low temperature. This is approximately one-half that determined for a 1.0-gram crab.

Data are available but have not been presented for the experimental temperature, 20° C. If any of the above comparisons are made at this temperature, the same trends are observed. They differ only in the fact that regression lines for the higher experimental temperature are located at a higher position on the ordinate. At this higher experimental temperature there is no evidence of heat depression, at either experimental salinity.

Hemigrapsus nudus: Comparison of the four acclimation temperatures at low salinity for *H. nudus* demonstrates that as acclimation temperature increases, oxygen consumption decreases (Fig. 6). There is observed a 105% increase for 1.0-gram crabs at the low temperature (5° C.) when this is compared with the high one (20° C.). The only combination of two lines which is not statistically significant is the comparison of the 10° and 15° C. acclimation temperature regression lines (Table I). All other combinations give a P value of less than one per cent.

When the slopes of the four acclimation regression lines are compared it is seen that there are no statistically significant differences (Table I). The four lines assume nearly parallel relations.

At a salinity of 75% sea water response of these crabs at the four acclimation temperatures is similar when compared with the lower salinity. As the acclima-
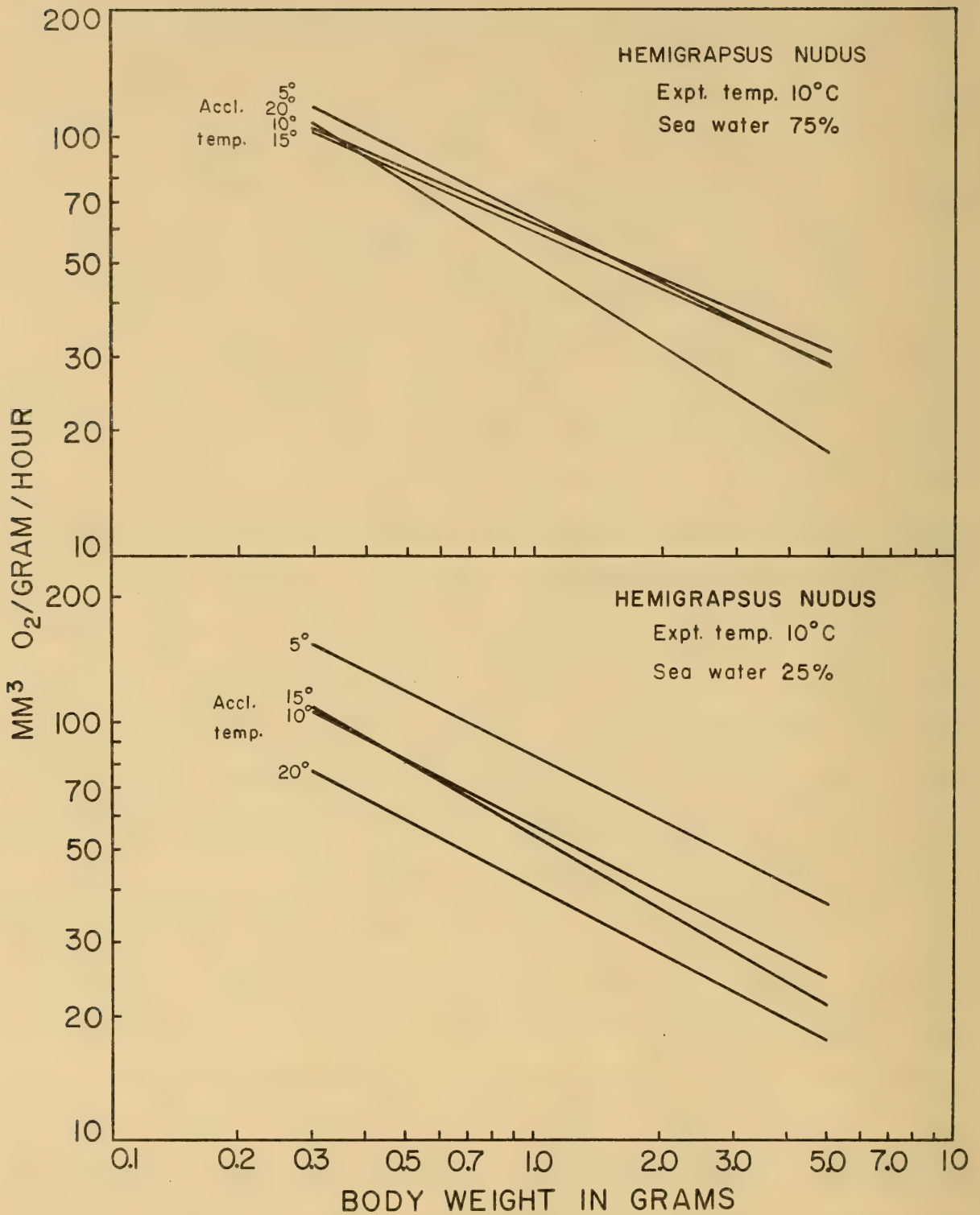
FIGURE 6. Effect of acclimation temperature on weight-specific oxygen consumption with increasing size in *Hemigrapsus nudus,* at the two acclimation salinities, 25% sea water (lower) and 75% sea water (upper). Regression lines were fitted by the method of least squares. Slope values, coefficients of correlation and other statistical data are given in Table I.

tion temperature increases, oxygen consumption again decreases over most of the weight range. Analysis of covariance of the total of the four lines shows a significant difference (P = 0.01). If a 1.0-gram crab is compared at high and low acclimation temperatures there is a 28% increase in oxygen consumption at the lower temperature. Further comparison of the 20° C. regression line with either the 10° C. or 15° C. line gives the same level of significance (Table I). If the 5°, 10° or 15° C. regression lines are compared within themselves, either there is no significance or a significance to the 5% level, a value considered to be statistically significant.

With regard to slope change at the higher salinity, the four acclimation temperature regression lines converge at the small end of the weight range (Fig. 6). Total line comparison for change in slope gives a significance to the 5 per cent level. This is true also for all combinations of the four regression lines with the exception of 10° and 20° C. where P = 0.01. There is an approximate 67% increase in weight-specific oxygen consumption for a 2.5-gram crab at 5° C. over that noted for the same weight at 20° C. This percentage increase is more than twice that observed for a 1.0-gram animal, when the same two acclimation temperatures are compared.

As in the case of *H. oregonensis* data are available but not presented for 20° C. experimental temperature for *H. nudus*. Again, the trends are the same, only the position on the ordinate differs.

Interspecific comparison: If the above results as determined by the four temperature acclimation regression lines are compared between species, at either salinity, it is observed that weight-specific oxygen consumption is similar in some instances. This is the situation at both experimental temperatures. For instance, at 25% sea water, 5° C. acclimation temperature a 1.0-gram *H. oregonensis* has an oxygen consumption per gram per hour of 87 mm.[3], and the same weight *H. nudus* with identical conditions has a value of 84 mm.[3] (3.5% difference). At 20° C. acclimation temperature, *H. oregonensis* has a value of 47 mm.[3] and *H. nudus,* 41 mm.[3] (15% difference). At 75% sea water, 5° C. acclimation temperature 1.0-gram *H. oregonensis* and *H. nudus* are about the same. At 20° C. there is a 35% difference, *H. nudus* having the higher rate (compare Figs. 5 and 6).

Effect of acclimation salinity

Hemigrapsus oregonensis: When weight-specific oxygen consumption is compared at low and high salinities at 10° C. experimental temperature and a given acclimation temperature, the higher rate is found at the low salinity (25%). Analysis of covariance of the total of the four acclimation temperature regression lines in relation to the two salinities shows a statistically significant difference (P = 0.01) between low and high salinity (Table II). At the 5° C. acclimation temperature (Fig. 7) a 1.0-gram crab shows a 29% increase at the low salinity over that for the same weight animal at the high salinity. Similarly, at the 20° C. acclimation temperature there is a 27% increase at the low salinity. In Figure 7 as well as Figure 8 only low and high acclimation temperatures with both salinities have been given. If either of the two intermediate acclimation temperatures

(10° and 15° C.) are compared at both salinities there is no significant difference (Table II).

There is a convergence of regression lines of each acclimation temperature at both salinities toward the higher end of the weight range. However, statistical comparison of the total acclimation temperature regression lines at the two salinities or comparison of any regression line, likewise at the two salinities, shows no significance with regard to change in slope (Table II). There is a tendency for the slope to decrease at the higher salinity for either the 5° or 20° C. acclimation temperature regression line.

TABLE II

Analysis of covariance of rates of oxygen consumption per gram per hour as a function of weight in Hemigrapsus oregonensis *and* H. nudus *at 10° and 20° C. experimental temperatures. Each acclimation temperature is compared at the two salinities, 25% and 75% sea water. P indicates the significance of the position of the regression lines on the ordinate. P_b indicates the significance of the change in slope of the regression lines*

Expt. temp. (°C.)	Accl. temp. (°C.)	P	P_b
	Total	0.01	N.S.
	5	0.01	N.S.
10	10	N.S.	N.S.
	15	0.05	N.S.
	20	0.05	N.S.
H. oregonensis			
	Total	N.S.	N.S.
20	5	N.S.	N.S.
	10	N.S.	N.S.
	15	N.S.	N.S.
	20	N.S.	N.S.
	Total	0.01	N.S.
	5	0.01	N.S.
10	10	0.01	N.S.
	15	0.01	N.S.
	20	0.01	0.05
H. nudus	Total	N.S.	N.S.
	5	N.S.	N.S.
20	10	N.S.	N.S.
	15	N.S.	N.S.
	20	N.S.	0.05

At the higher experimental temperature (20° C.) when any of the four acclimation temperature regression lines are compared at both salinities (5° C. at 25% and 75% sea water) there are no statistically significant differences (Table II). Similarly, there are no significant slope changes under these experimental conditions.

Hemigrapsus nudus: Consideration of the data for this species is quite different from that described for *H. oregonensis*. Weight-specific oxygen consumption measured at 10° C. experimental temperature is higher when crabs are acclimated to the low temperature, low salinity combination when compared with low temperature, high salinity (Fig. 8). Covariant analysis of the total four acclimation

temperature regression lines relative to the two salinities gives a significant difference (P = 0.01). A 1.0-gram crab at 25% sea water shows a 31% increase over that same weight crab at 75% sea water. The lines from which this weight crab was taken are significant at the one per cent level (Table II). At the higher acclimation temperatures (Fig. 8 and Table II) crabs acclimated to the higher salinity have a higher rate of oxygen consumption and the differences at each acclimation temperature for both salinities are statistically significant (P = 0.01). Comparison of a 1.0-gram crab acclimated to 20° C. and both salinities shows a 25% difference in rate of oxygen consumption.
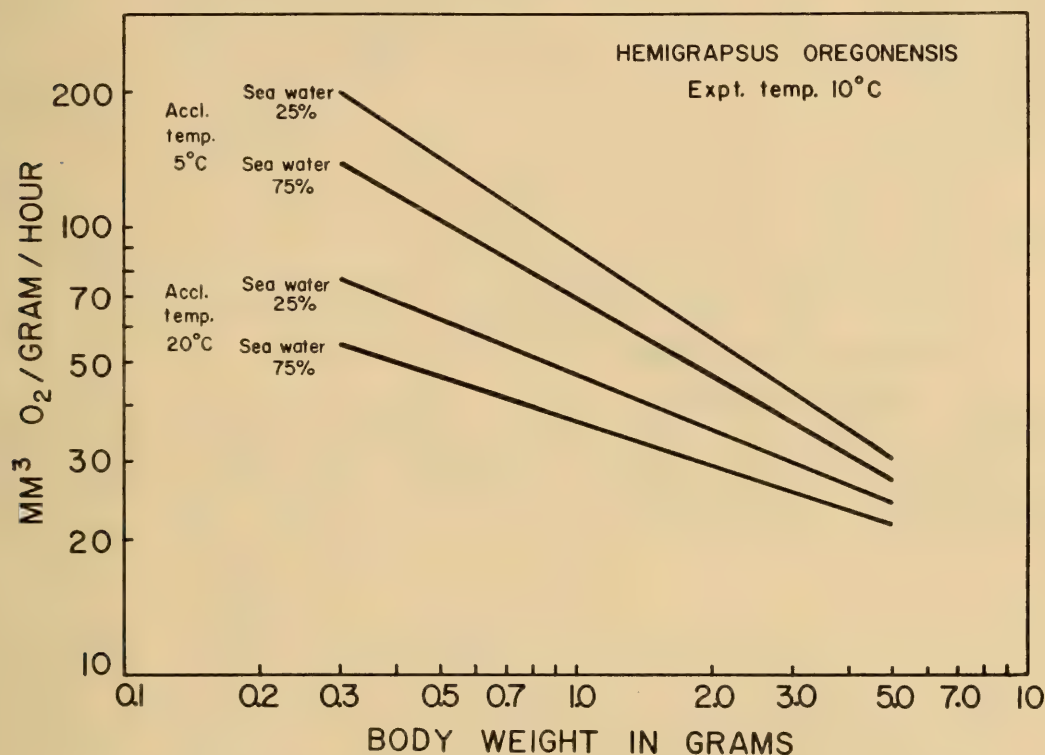

FIGURE 7. Effect of acclimation salinity on weight-specific oxygen consumption with increasing size in *Hemigrapsus oregonensis* at two acclimation temperatures (5° and 20° C.). Regression lines were fitted by the method of least squares. Slope values are given in Table I, significance of slope change and other data are given in Table II. The two intermediate acclimation temperatures (10° and 15° C.) are not included but the analyses are given in the tables.

Reference to Figure 8 and Table II indicates no significant changes in slope either for a given acclimation temperature regression line compared at two salinities or total regression lines.

The response of this species at the 20° C. experimental temperature shows no statistically significant differences when any acclimation regression line is compared at the two salinities, or when slopes are compared (Table II).

Interspecific comparison: When these two species are compared with regard to their acclimation response to low and high salinity, one basic difference is noted. When both species are acclimated to low temperature, low salinity and low temperature, high salinity the weight-specific oxygen consumption regression lines
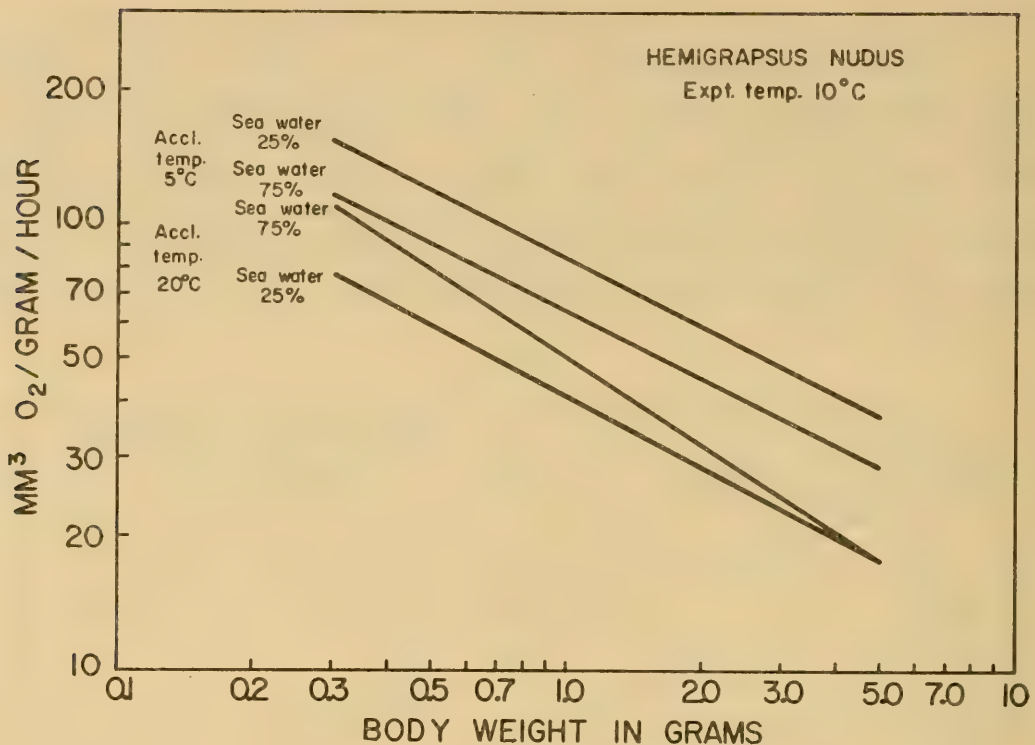
FIGURE 8. Effect of acclimation salinity on weight-specific oxygen consumption with increasing size in *Hemigrapsus nudus* at two acclimation temperatures (5° and 20° C.). Regression lines were fitted by the method of least squares. Slope values are given in Table I, significance of slope change and other data are given in Table II. The two intermediate acclimation temperatures (10° and 15° C.) are not included but the analyses are given in the tables.

are similar in position and slope. Low temperature, low salinity combination gives the greater rate. If, however, crabs are acclimated to high temperature at both low and high salinities, *H. nudus* has the higher rate at the high salinity combination and *H. oregonensis* at the low salinity combination. Percentage differences of a 1.0-gram animal for both species are approximately the same, 25%.

Effect of size

Hemigrapsus oregonensis: Reference to Figure 5 and Table I shows that there is a statistically significant change (P = 0.01) in slope (decrease) as the acclimation temperature increases, when crabs are measured at 10° C. experimental temperature, and maintained at the low acclimation salinity. The fact that the four regression lines converge at the higher end of the weight range shows that weight-specific oxygen consumption of small animals, except at the low temperature, is affected by increase of acclimation temperature to a greater degree than large ones. With increasing weight there is a smaller change in rate for 20° C. acclimated animals than for 5° C. ones. Thus, animals acclimated to high temperatures are less size-dependent. The Q_{10} values increase as weight increases over the range from 5° to 10° C. Comparison of a small crab (0.8-gram) with a large one (3.0-gram) shows a Q_{10} change from 1.9 to 2.4. The Q_{10} decreases with weight over temperatures from 10° to 20° C.

At the high salinity, analysis of covariance of the four acclimation temperature regression lines shows no statistical significance relative to slope change. As a result no real size effect is demonstrable (Fig. 5). The Q_{10} values at the 5° to 10° C. temperature range increase as weight increases; Q_{10} of 0.8-gram crab is 1.6, of a 3.0-gram crab, 1.9. An inverse Q_{10} relationship with weight or no change is noted at the two other temperature comparisons.

When the two acclimation salinities are compared at each acclimation temperature, relative to slope change no statistically significant differences are found (Figs. 7, 9 and Table II). Figure 9 compares different weight animals at the two salinities and over the range of acclimation temperatures. Small crabs (0.8-gram) and large ones (3.0-gram) have been chosen from the regression lines in Figure 5. If the per cent change (increase) in the rate (oxygen consumption/gram/hour) is determined for the two salinities at each acclimation temperature the following values obtain. For small crabs at 5° C. there is a 30% change in rate; 10° C., 16%; 15° C., 46% and 20° C., 23%. For large crabs (3.0-gram) the per cent change (increase) is less: 5° C., 17%; 10° C., 7%; 15° C., 4%; 20° C., 20%. It is noted that for each acclimation temperature the
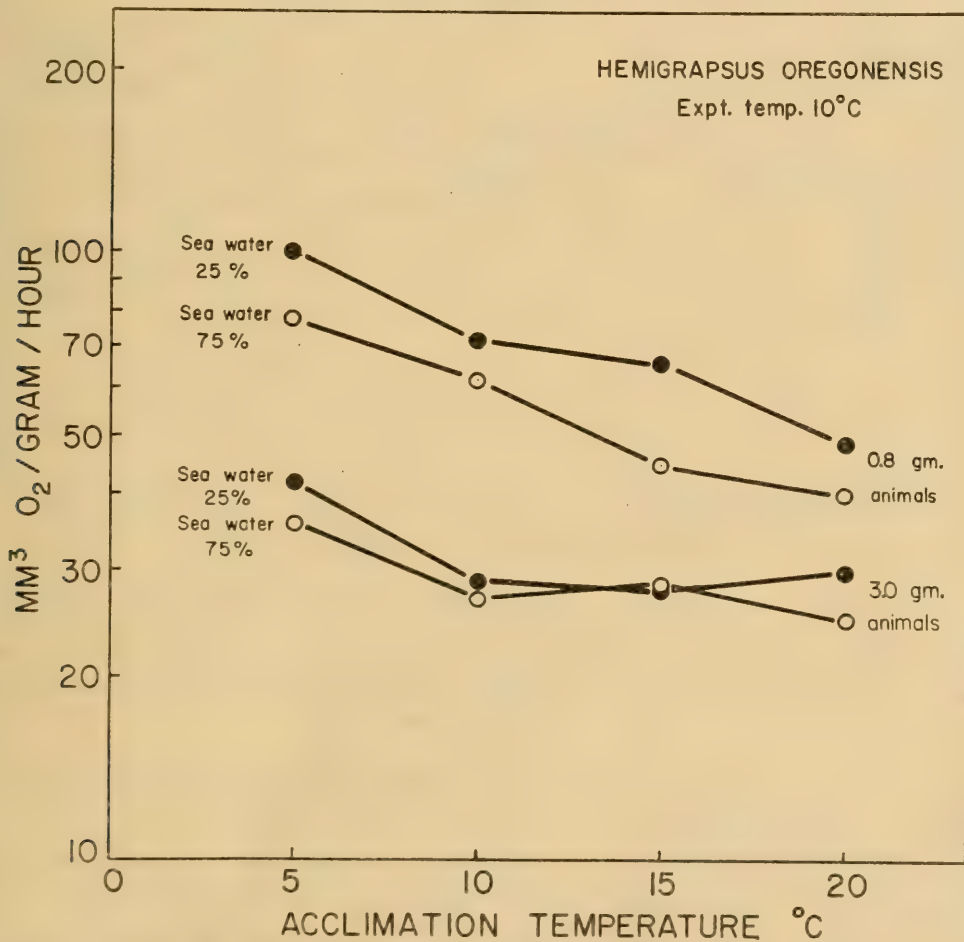

FIGURE 9. Effect of size on weight-specific oxygen consumption in *Hemigrapsus oregonensis* at the two acclimation salinities (25% and 75% sea water) and over the range of acclimation temperatures (5°, 10°, 15° and 20° C.). These weights were chosen from regression lines fitted by the method of least squares for data shown in Figure 5. The statistics for the size effect are given in Tables I and II.

rate for the low acclimation salinity is higher generally than that recorded for the high salinity.

Hemigrapsus nudus: For this species, at the low salinity, comparison of the total four acclimation temperature regression lines shows no significant slope change (Fig. 6 and Table I). Comparing Q_{10} values at the temperature interval of 5° to 10° C. shows no increase in Q_{10} as weight increases; Q_{10} of an 0.8-gram animal is 2.2; 3.0-gram crab, 2.3. Over the range from 10° to 15° C., there is only a slight
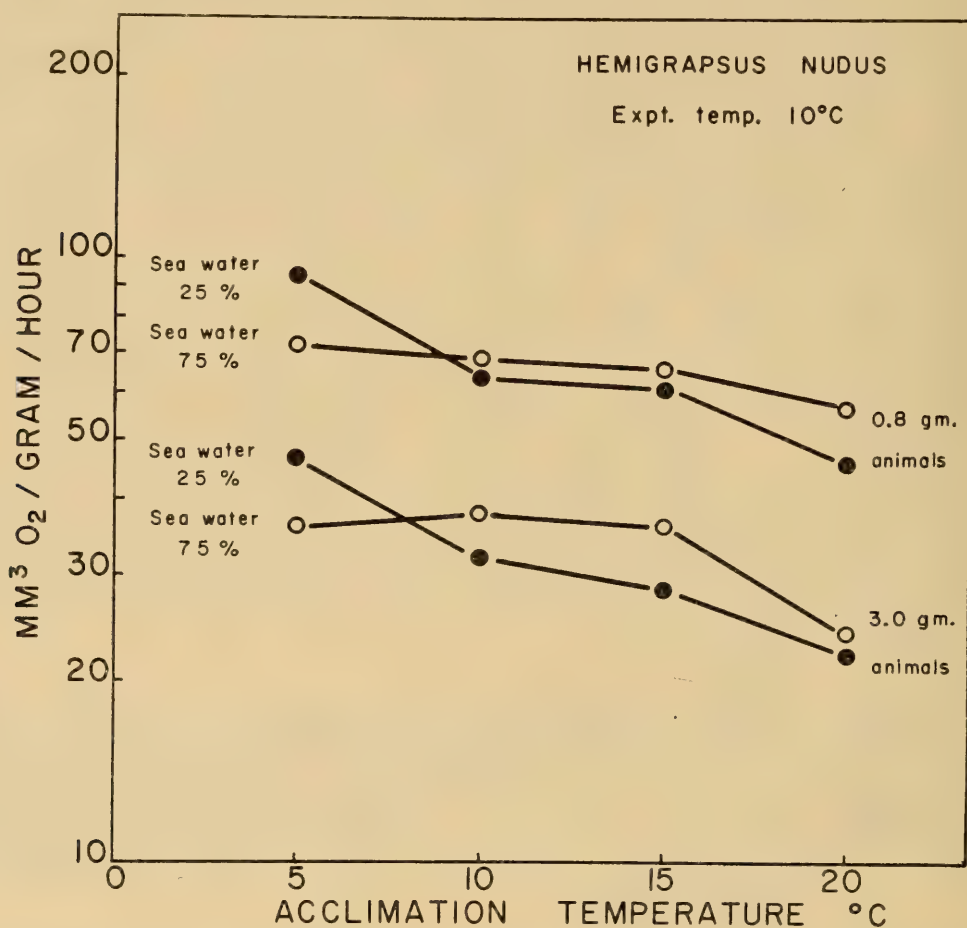

FIGURE 10. Effect of size on weight-specific oxygen consumption in *Hemigrapsus nudus* at the two acclimation salinities (25% and 75% sea water) and over the range of acclimation temperatures (5°, 10°, 15° and 20° C.). These weights were chosen from regression lines fitted by the method of least squares for data shown in Figure 6. The statistics for the size effect are given in Tables I and II.

change, 1.1 to 1.3, as there is observed over the range from 15° to 20° C., 1.8 for small crabs to 1.6 for large ones.

Covariant analysis of total four acclimation temperature regression lines at the high salinity gives a significance in slope change at the five per cent level (Fig. 6 and Table I). In this instance, however, the four regression lines converge to the left (small weights) and this demonstrates that large animals are affected to a greater extent as acclimation temperature increases. With increasing weight there is a greater change in rate for 20° C. acclimated animals than for 5° C. ones. Animals acclimated to these higher temperatures are more size-dependent. If

Q_{10} values are compared over the range from 5° to 15° C. there is no increase with weight. Over the range from 15° to 20° C., the increase is direct with size, 1.3 (0.8-gram) to 2.2 (3.0-gram).

No statistically significant differences in slope exist when weight-specific oxygen consumption for total lines is compared for both salinities at each acclimation temperature (Figs. 8, 10 and Table II). It is noted, however, that a five per cent level of significance results when rate of oxygen consumption is compared at 20° C. acclimation temperature. Again as with *H. oregonensis* small (0.8-gram) and large (3.0-gram) crabs have been chosen from the regression lines in Figure 6 and presented in Figure 10 for comparison. The per cent change (increase) in the weight-specific oxygen consumption results in the following values. For small crabs at 5° C., there is a 31% change in rate; 10° C., 10%; 15° C., 8% and 20° C., 24%. For large crabs the per cent change is, 5° C., 28%; 10° C., 19%; 15° C., 29% and 20° C., 9%.

Interspecific comparison: It can be stated generally that increase in acclimation temperature affects size differentially at low salinity in *H. oregonensis,* whereas this effect is not noted in *H. nudus.* At the higher salinity *H. nudus* shows a differential size effect, whereas *H. oregonensis* does not. When the two salinities are compared at any acclimation temperature no size effect is noted for either species. If Q_{10} values are compared, there is a general increase with weight increase in both species at the low salinity over the range 5° to 10° C. At the two higher temperature intervals generally there is a decrease in Q_{10} with size or essentially no change. At the higher salinity, over the temperature range 5° to 10° C., Q_{10} values for *H. oregonensis* increase with size, whereas there is no change for *H. nudus.* For the two higher temperature ranges Q_{10} values either decrease or remain the same for *H. oregonensis,* and increase or remain the same for *H. nudus.* For any weight crab at either salinity, Q_{10} values tend to decrease as temperature increases. An exception to this is recorded for *H. nudus* at high salinity where Q_{10} increases as the acclimation temperature increases.

Reference to Figures 9 and 10 shows that at the low acclimation temperature rate of oxygen consumption for large and small animals of both species is always higher for ones acclimated to low salinity. As acclimation temperature increases, low salinity continues to result in a higher rate for *H. oregonensis,* but for *H. nudus* higher salinity has a greater effect.

DISCUSSION

Seasonal rate-temperature experiments

Oxygen consumption measurements made on both species of crabs over a range of temperatures, summer and winter, and kept at the seasonal salinity, show that summer crabs, for any weight animal, have a higher respiratory rate than winter ones over the physiological temperature range. Further, heat depression occurs for winter animals at approximately the same high temperature as for summer crabs, and winter animals extend the range of low temperature to a point below that for summer ones. These responses result in a greater temperature range for winter animals before temperature depression occurs. The data relate to a condition described by Precht (1951) as inverse compensation (type 5). The

seasonal rate-temperature curves do not correspond to the frequently reported relationships, winter rates higher than summer (partial compensation, type 3).

Hemigrapsus oregonensis: Use of the acclimated rate-temperature curve has been suggested as a method for comparing species ecologically, and for determining whether acclimation to low temperatures shows proportionally a greater compensation when compared with high temperatures (Bullock, 1955). An acclimated rate-temperature curve can be plotted on Figure 3. This curve is determined by noting weight-specific oxygen consumption for winter animals at 5° C. (30 mm.^{3} O_2/gram/hour) with summer ones at 20° C. (72 mm.^{3}). These two points represent animals seasonally adapted to their own approximate natural temperatures and measured at those temperatures. This acclimated rate-temperature curve for *H. oregonensis* has a higher Q_{10} than either the summer or winter acutely measured curves. This means that animals show a greater temperature dependence when adapted to their field temperatures than when measured acutely over a series of temperatures, and before any demonstrable acclimation occurs. Bullock (1955) states that the acutely measured curve is steeper than the acclimated one (more temperature-sensitive). The curves for this species show this not to be the case, and if the acclimated rate-temperature curve is significant, then Q_{10} may have no real meaning.

A further point of comparison is weight-specific oxygen consumption for summer animals at 5° C. and 30° C., and the same comparison for winter crabs at these two temperatures. Summer animals have the same rate at the low and high temperatures, and winter ones are similar. If summer and winter animals are compared together at either the low or high temperature, again their rates are nearly the same. Summer and winter animals are depressed about the same at the high temperature and no depression occurs at 5° C. These data, coupled with the acclimated rate-temperature curves, are good evidence to show that no acclimation has resulted.

Hemigrapsus nudus: When an acclimated rate-temperature curve is plotted for this species (Fig. 4) the rate for winter animals at 5° C. is 20 mm.^{3} O_2/gram/hour, and for summer animals at 20° C., 83 mm.^{3}. This curve is steeper (higher Q_{10}) than the winter acutely measured rate-temperature curve, and is approximately the same as the summer curve. Thus, winter animals show a greater temperature dependence when adapted to their field temperatures. Temperature dependence for summer animals is about the same for acclimated and acutely measured rates.

When weight-specific oxygen consumption is compared for summer or winter animals at 5° and 30° C., both have a higher rate at the high temperature. But, if summer and winter crabs are compared at 30° C., both have the same rate, the depression being similar. Again this evidence shows that no seasonal acclimation has been found.

Metabolism and size

Relationship of body size to metabolism is not only recognized but is invariably the subject of controversy when this dependence is discussed in various animal groups. The general concept states that weight-specific oxygen consumption is higher for small animals when compared with large ones, measured at a given tem-

perature and determined for animals of a given species or for closely related ones. If the logarithm of the rate is plotted as a function of the logarithm of weight a linear relation exists, with a negative regression coefficient. If total oxygen consumption is plotted against body weight, large animals have a higher metabolic rate. These relationships have been shown to exist for poikilotherms and homiotherms. Absolute changes occur when intra- and interspecific comparisons are made relative to (1) temperature, (2) displacement of populations latitudinally and vertically, (3) season, (4) sex, (5) state of nutrition, (6) age, (7) other extrinsic and intrinsic factors. Relative changes remain, however, as mentioned above. Whether one is concerned with weight-specific or total oxygen consumption the problem involves the dependence of the rate on the weight, namely, the value and significance of the power function,

$$\frac{O_2}{W} = aW^{b-1} \quad \text{or} \quad O_2 = aW^b$$

The regression coefficient employed frequently is 0.67, a value that corresponds to the relationship between the surface of an animal and its body weight. This relationship indeed appears to be undoubtedly a spurious correlation as suggested by Weymouth, Crismon, Hall, Belding and Field, (1944); Brody (1945); Zeuthen (1953) and others. Regardless, it would appear ludicrous to assume the validity of such a "standard" value, particularly in view of the fact that rate functions vary with the influence of internal and external parameters. Further, there is no reason to believe that this influence on rate merely shifts the position of the regression line on the ordinate. Zeuthen (1953) has shown that the regression coefficient, b, assumes very different values ranging from 1.0 to negative numbers. Weymouth *et al.* (1944) compared respiratory rate to body weight in the kelp crab *Pugettia producta* with a series of Crustacea and found an exponent of 0.80. Scholander *et al.* (1953) likewise found a similar exponent, 0.80 to 0.85 when arctic and tropical crustaceans and fish were compared. Roberts (1957a) found for *Pachygrapsus crassipes* a power function of 0.664. Bertalanffy (1951) has correlated three different proportions of metabolism to weight with three growth types, namely ⅔, ¾ and 1. Categorical definition of these power functions suggests them to be species-specific and unalterable, at least with respect to metabolic relationships. Bertalanffy and Krywienczyk (1953) demonstrated that oxygen consumption in brine shrimp, *Artemia salina,* plotted as a function of body weight follows the surface rule. These animals were cultured in artificial sea water and maintained at 25° to 27° C.

Another aspect of weight-specific respiration to body weight is the change in slope when two or more regression lines are compared, these lines resulting from measurements made under different seasonal or experimental conditions (altering one or more parameters, such as a constant temperature and two salinities). This concerns the significance of parallelism or convergence of regression lines at the low or high end of the weight range. These conditions can be discussed either by statistical analysis of b values or by comparison of Q_{10}. As regards the latter, Rao and Bullock (1954) have reviewed much of the literature. They summarize generally, stating (p. 38) that there is "a common increase of temperature coefficient with size, on the specified assumption of a weight regression." Ellenby (1951)

compared body size in the isopod *Ligia oceanica*, relative to oxygen consumption and pleopod beat. He found that total oxygen consumption at 25° C. was proportional to the 0.726 power of body weight, but that this value was not statistically different from 0.66. Oxygen consumption per unit of length² is constant over the size range (surface area is proportional to length²). Pleopod beat, on the other hand, gave a 0.66 power of body length at 15° C. and at 25° C. the value was 0.59, and these are not significantly different from the 0.5 power. In a later paper on a study of predicting oxygen consumption, Ellenby and Evans (1956) believed that in *Ligia* greater accuracy could be obtained from body weight than from a function of body length. Oxygen consumption prediction for prepupae of *Drosophila melanogaster* was found to be more accurate if based on surface area. Vernberg and Gray (1953) studied oxygen consumption of brain brei determined at 30° C. in a series of marine teleosts, and found two species whose rates of oxygen consumption were independent of weight or length.

Clark (1955) studied the effect of temperature on the oxygen consumption of the terrestrial amphipod *Talitrus sylvaticus*. He reported an exponent of 0.836 at 25° C. Further he showed that weight-specific oxygen consumption was greater in small animals (1.5 mg.) than in large ones (21.0 mg.) at temperatures above 15° C. The Q_{10} values for winter animals between 20° and 30° C. was 2.33 for small amphipods and 1.66 for large ones. Roberts (1957a) determined oxygen consumption in the shore crab *Pachygrapsus crassipes* that were acclimated to three temperatures for a period up to seventeen days. Over the weight range used (1 to 40 grams) he found that Q_{10} varied directly with weight over the acclimation temperature range 16° to 23.5° C. ($Q_{10} = 2.73$ at five grams, to 3.24 at thirty-five grams). No change was noted between 8.5° and 16° C. (Q_{10} for all weights was 2.66). At the higher acclimation temperature, slope of the negative linear regression changed from − 0.336 at the two lower temperatures to − 0.270 at the high temperature, a change shown to be statistically significant.

If Q_{10} values for seasonal rate-temperature data are compared for the two species of *Hemigrapsus* and for any weight of crab it is seen that generally Q_{10} values are relatively low at low temperatures in winter animals. A rise with increasing temperature to approximately 15° C. occurs, then Q_{10} values decrease as the temperature continues to rise. The same pattern exists for summer crabs except that at the lowest temperatures (3.5° to 5° C.) there is cold depression. When different weight animals are compared at any temperature interval, no generalization can be made as no trend is noted for either species, summer or winter.

Experimental results for the series of acclimation temperature and salinity combinations fail to show any definite trend of Q_{10} values with increase in size or with increase in temperature for a given size. It should be mentioned that as with seasonal rate-temperature experiments, there is a tendency for Q_{10} values to increase at lower acclimation temperatures at both salinities, and then to decrease as acclimation temperatures increase. It is well to note at this point that Rao and Bullock (1954) have shown that in many instances Q_{10} shows no trend with size. These data support this contention.

Dependence of metabolism on body size for the temperature and salinity acclimation studies is somewhat at variance with that reported in the literature. Weight-specific oxygen consumption *b* values for both salinities and species range

from − 0.685 to − 0.333, or the positive linear regression coefficients range from 0.315 to 0.667 (Table I). Only a very few instances approached the reported 0.66 or 0.75 exponent. For the most part positive regression coefficients are relatively low values. If individual slopes or total comparison of the four acclimation temperatures at either salinity are noted, it is seen that these slopes change in some cases to a considerable degree. Statistically significant differences have been noted for *H. oregonensis* at low salinity, and *H. nudus* at high salinity. For any given acclimation temperature, scatter tends to be greatest for small crabs. As the acclimation temperature increases scatter increases along the entire weight range. Reference to Table I gives the correlation coefficients, *r,* of each of the acclimation temperature regression lines for both salinities and both species. In each case the value for *r* is significant at the one per cent level as determined from a table of significance of *r* (Simpson and Roe, 1939). Sample size (N) is greater than 25 for all calculated lines. It should be mentioned that regardless of increased scatter at the small end of the weight range, a straight line has been demonstrated to be statistically the best fit, as a parabolic function described less well weight-specific oxygen consumption data.

From these data it would be difficult to assign a positive linear regression coefficient to a species and suggest that such a value might be an inherent or fixed character of that species. It is evident that the regression coefficient is dependent upon past environmental histories of the animal as well as experimental variables (temperature and/or salinity) to which the animal is exposed. Variability of the response of an animal is noted to be different depending upon the weight range, temperature, particularly, and salinity combinations.

Temperature and salinity relations

In a recent paper Kinne (1956a) has discussed aspects of temperature and salinity and their biological effect on marine, brackish and fresh water animals. Generally, he believes that temperature increase intensifies activity and decreases resistance, and temperature decrease produces the opposite effect. Further, marine organisms often resist a low salinity at extremely low temperatures. The resistance and existence of marine and brackish species is facilitated in a high salinity by a relatively high temperature, and in a low salinity by a relatively lower one. Near limits of tolerance, low salinity and high temperature are often lethal, but low temperature and low salinity are tolerated. Heat resistance in brackish water species depends to a great degree on salinity. Decrease in environmental salinity causes a decrease in heat resistance, and with an increase in salinity, heat resistance increases.

Results from experiments on the effect of temperature and salinity on high temperature tolerance in the two species of *Hemigrapsus* do not support completely these ideas. It has been determined that acclimation to high temperature resulted in an appreciable increase in temperature tolerance, both in summer- and winter-adapted animals, and acclimation to low salintiy decreased resistance. In this geographical area the two experimental combinations of temperature and salinity that produced the greatest (20° C., 75% sea water) and least (5° C., 35% sea water) resistance to high test tolerance temperatures never occur seasonally in the environment (Todd and Dehnel, 1960).

Numerous studies have demonstrated that metabolism increases when organisms are removed from their normal salinity medium and placed in a stress medium. Schlieper (1929) showed that oxygen consumption of the crab *Carcinus maenas* increased with decreasing salt concentration. Similarly, results were obtained for *Nereis diversicolor* and gill respiration of *Mytilus edulis*. Schwabe (1933) has reported a similar situation for the crayfish, *Potamobius fluviatilis,* as have Flemister and Flemister (1951) for the crab *Ocypode albicans*. These reports concern measurements made in different salinities and at a given temperature. It has been suggested that this increased rate of oxygen consumption reflected increased osmotic work. Schlieper (1929) suggested that in higher salt concentrations CO_2, a general stimulant for cellular respiration, would be removed more readily from the animal, whereas in lower salinities there would be a tendency for CO_2 to accumulate, thus increasing respiratory rate. Later, Schlieper (1935) proposed the idea that water content of tissues was related directly to the amount of oxygen consumed. A higher water content increased volume of the tissues and the surface, which in turn facilitated absorption and hence the consumption of oxygen. This interpretation that increased metabolic rate results from increased osmotic stress has been subject to criticism. Krogh (1939) has discussed thoroughly this aspect of osmoregulation. Wikgren (1953) in an extensive review of osmoregulation as it is influenced by temperature has stated that the difference in oxygen requirement in fresh water as opposed to isotonic media was not attributable to osmotic regulation. He suggests that low salt concentrations stimulate basal metabolism directly by increased swelling of tissues or by influencing endocrine balance. These ideas in part are in accord with Schlieper (1935).

Gross (1957) reports that certain crabs, *e.g., Pachygrapsus,* show violent attempts to escape from a medium which departs much from normal sea water in concentration. He suggests that increased oxygen consumption in increased osmotic stress results from increased muscular activity. *Pachygrapsus crassipes* has been shown by Gross (1957) to be a good regulator in both hypo- and hypertonic media. This species lives in an intertidal situation in sea water of a concentration of approximately 100%, and probably never is exposed to lower salinities in the field.

Considering the results obtained in this study, it is evident that when *H. oregonensis* and *H. nudus* are acclimated to a series of temperatures at either low or high salinity, weight-specific oxygen consumption is greatest after acclimation to the low temperature (5° C.). As that acclimation temperature increases, oxygen consumption decreases. When low and high salinities are compared at each acclimation temperature, the respiratory rate for *H. oregonensis* is higher at the low salinity, over most of the weight range for all temperatures (see Figure 7 for 5° and 20° C.). For *H. nudus* acclimated to the same conditions, the rate is higher for the low temperature, low salinity combination, but as the acclimation temperature increases, oxygen consumption is higher at the high salinity (Fig. 8). The results obtained for *H. oregonensis* might be considered to be in accord with the idea of escaping an unfavorable medium as suggested by Gross (1957), or due to increased osmotic work at lower salinities as suggested by Schlieper (1935) and others. On the other hand these ideas would not explain the results obtained for *H. nudus*. When seasonal environmental changes in this area are considered, it cannot be stated that one temperature-salinity combination is normal any more

than the reverse relations that occur at another season. This is in contradistinction to that for an open coast intertidal species. It is recalled that during the summer a low field salinity normally exists, comparable to the experimental one, and is maintained for several months. During this period the temperature is high. The animals on which these experiments were conducted were summer-adapted ones. Further, both species are exposed to the same environmental conditions in the field. In addition, animals returned to the laboratory and placed directly into low salinity sea water have never been observed to attempt to escape from that water. There has been no evidence of this at any of the acclimation temperatures with the combination of low salinity. This is also the situation for winter-adapted crabs, collected from a high field salinity and low temperature and placed either directly or gradually into low salinity water in the laboratory. Under the circumstances of summer field salinities, it seems highly improbable that the higher rate of oxygen consumption at the low salinity is the result of the attempt by these crabs to escape this low concentration of sea water, particularly since this low salinity is normal for several months of the year. *A priori,* one would expect both species to approximate the same respiratory rate, even perhaps *H. nudus* to have a somewhat higher rate. This is based on the fact that the conditions to which *H. nudus* are exposed in this locality, are the unusual ones when the ecology of this species is recalled over the latitudinal distribution. *H. nudus,* for the most part, is an intertidal species and occupies a habitat somewhat comparable to *Pachygrapsus crassipes.*

In connection with the observed high metabolic rate at low salinity, the work of Schlieper (1953, 1955, 1957) should be noted. Weight-specific oxygen consumption was determined for gills of *Mytilus edulis* collected from high salinity water (North Sea 30‰) and for ones from the same area but acclimated to low salinity (15‰) for four weeks. Respiration was higher at the low salinity by nearly a factor of 2. *Mytilus* living in low salinity water (Baltic Sea, 15‰) had a higher oxygen consumption also by a factor of approximately 2, than ones collected from the Baltic but acclimated for four weeks to high salinity water (30‰). Further, weight-specific oxygen consumption for animals acclimated to low salinity was approximately the same as that recorded for ones normally living in low salinity water, about 140 ml. O_2 per gram per hour. High salinity respiratory rate for both instances was about 80 ml. O_2 per gram per hour. This instance serves to demonstrate the fact that tissues of a *sessile* animal adapted to low salinity water have a higher respiratory rate when compared with ones adapted to high salinity water. Further, laboratory acclimation to low salinity results in a higher rate. On the other hand, measurements of mechanical activity of gill cilia, frequency of heart beat and heat resistance are lower in low salinity sea water.

Differential metabolic response of *Hemigrapsus* to salinity is believed, in part, to be the result of osmotic stress, *i.e.,* increased work to maintain an osmotic balance. At high temperatures metabolic activity increases and low salinity may be a greater stress than a high one. This low salinity could reduce resistance and cause a greater osmotic problem; the resulting high oxygen consumption reflects osmotic work. The problem may be resolved, in part, by a present study of osmotic behaviour of these two species to determine the responses to field conditions and to various laboratory combinations of temperature and salinity. Death at high temperature and low salinity may be due to osmotic breakdown. But the high temperature at which osmotic breakdown occurs depends upon the acclimation

temperature, salinity and season. Gross (1957) has calculated data from Jones (1941) to demonstrate change in osmotic gradients between blood and external medium in *H. oregonensis* and *H. nudus* as increased osmotic stresses are applied. These data show that as sea water concentration decreases below normal sea water, the internal osmotic concentration remains relatively constant.

It is of significance and interest to be able to compare different populations of the two species of *Hemigrapsus*. Gross (1955) has demonstrated the general ability of terrestrial and semi-terrestrial crabs to regulate osmotically in dilute and concentrated sea water. He has found that *H. oregonensis* and *H. nudus* from southern California can regulate in 150% sea water for about twenty hours. However, both species from Vancouver die at that concentration in from two to six hours. At 125% sea water survival is longer, one to two weeks, but mortality is relatively high during this time.

Recently Verwey (1957) has reviewed the problem of the influence of temperature on osmoregulation and has discussed means whereby marine and brackish animals attempt to live in a changing environment. For instance, the shrimp *Crangon crangon* migrates seaward toward the North Sea in autumn and returns in the spring to the region of the Dutch coast. Experimentally, it was found that salinity must increase as temperature drops for survival of this species. By migrating seaward the shrimp reach waters of higher salinity and somewhat warmer waters for winter survival, and return in the spring when coastal waters have a higher salinity. Other species that show a seasonal migration are those that migrate offshore in spring and return in autumn, such as the spider crab *Hyas araneus,* the shrimp *Crangon allmanni* and the prawn *Pandalus montagui*. This suggests that low salinities are tolerated better when the temperature is low. Still other species are those that do not migrate, the crab *Rhithropanopeus harrisi* and the amphipod *Gammarus duebeni*. These forms withstand low salinity better with a corresponding low temperature. They differ completely from *C. crangon* in their tolerance to temperature and salinity, are similar to *Hyas araneus* in tolerance, but differ from *H. araneus* in that they do not migrate. Verwey, in discussing these data, calculated changes in osmotic pressures expressed in atmospheres as opposed to the usual method of expressing salinity data as parts per thousand, freezing point depression (delta) or per cent sea water. Expression in atmospheres relates pressure, salinity and temperature. For instance, *C. crangon* kept in a salinity of 33‰ and given a temperature change from 4° C. to 21° C., gives a value of 19.0 atmospheres for the blood at 4° C., and 19.1 atmospheres at 21° C. Sea water of 33‰ at 4° C. has an osmotic pressure of 22.99 atmospheres and at 21° C. the pressure is 24.40 atmospheres. Osmotic pressure of the blood is unchanged, sea water osmotic pressure has increased with the rise in temperature, and the gradient between blood and sea water has increased. A salinity of 33‰ is optimal for adult *Crangon* at 4° C. At a higher temperature (21° C.) the optimal salinity is about 28‰. The absolute differences expressed in atmospheres in osmotic pressure of the medium and blood at the optimal salinities for these two temperatures are the same (see Verwey, 1957, for further discussion and references). Migration of *Crangon* in the autumn results in the animal moving into water of a higher salinity and hence higher osmotic pressure, eliminating differences between blood and external medium caused by the drop in temperature, as well as the drop in salinity. It is suggested that *Crangon* attempts to maintain a

constant value in the differences between osmotic pressures of blood and medium by this migration. In connection with these aspects Panikkar (1940, 1941) found similar relationships for prawns and points out that minimum blood osmotic pressure can be lowered as temperature increases and thus osmotic work to maintain hypertonicity is less at high temperatures. He has suggested that colonization by marine animals of fresh and brackish waters in the tropics may be explained by the fact that range of tolerance to lowered salinity increases with higher temperatures. Euryhaline species can maintain hypertonicity readily and stenohaline or slightly euryhaline marine species can cope with brackish waters when temperatures are high.

The ideas suggested by Panikkar (1940, 1941) might be extended to help explain the distribution locally of *Hemigrapsus,* particularly *H. nudus.* Both species, as noted previously, breed in late winter and spring. The earliest breeding occurs during the period of low temperature and high salinity. As breeding proceeds, temperature-salinity relations change until summer conditions exist. Water currents and tides are such that zoea and megalops larvae from regions near the outer coast could be carried through the Strait of Juan de Fuca, into the Strait of Georgia. Temperature-salinity field conditions are approaching summer ones as the larvae are released by the female and become planktonic. As larvae enter the Strait of Georgia, Fraser River water is mixing with the more saline water of the Strait, and the larvae are in a low salinity environment. However, sea water temperature is rising, and with increasing temperature, tolerance to low salinity increases. Thus, perhaps larvae when exposed to low salinity, resist its effect, survive and establish themselves in an area that seasonally has low salinity and high temperature conditions. Such conditions as these might explain the invasion of *H. nudus* into this geographic area. It would appear that *H. oregonensis* occupied this area originally, and that secondarily *H. nudus* became established. Such an idea is based on the fact that the usual ecological environment of *H. nudus* is the open coast intertidal. If an east-west cline of intertidal distribution is considered, *H. nudus* is very abundant intertidally, whereas the density of the *H. oregonensis* population decreases as open coast areas are reached.

Experiments with the larvae of these two species, to determine resistance, would clarify the responses to high test tolerance temperatures, when exposed to various temperature-salinity combinations. Preliminary experiments suggest that larval responses are similar to those of the adults, and this relates favorably to environmental conditions that exist during the planktonic and settling period of *Hemigrapsus* larvae. It would be most significant to determine acclimation of oxygen consumption and temperature tolerance to different temperature-salinity combinations for populations of both species throughout their latitudinal distribution.

SUMMARY

1. Oxygen consumption measurements have been made on two species of crabs, *Hemigrapsus oregonensis* and *H. nudus.* These crabs were either brought from field conditions and measured directly, or acclimated to different combinations of temperature and salinity for two to three weeks prior to experimentation.

2. Acutely measured seasonal rate-temperature curves for summer and winter animals of both species, kept at their seasonal salinity, have shown that summer

animals at all temperatures over the physiological range have a higher weight-specific oxygen consumption than winter ones.

3. Winter animals of both species are depressed at a lower temperature than summer ones, and depression at the high temperature begins at about the same point for both summer and winter crabs. Comparison of summer and winter animals of both species at 30° C. shows about the same amount of depression.

4. Both species fail to show an acclimation shift on the ordinate, summer versus winter, but winter animals show an acclimation of the upper physiological limit.

5. Acclimated rate-temperature curves for both species generally have a higher Q_{10} than either the summer or winter acutely measured curves.

6. When summer crabs of both species are acclimated to a series of temperatures (5°, 10°, 15° and 20° C.) at either low salinity (25% sea water) or high salinity (75% sea water), it is seen that as the acclimation temperature increases, oxygen consumption decreases over most of the weight range. Statistical analysis shows these changes in ordinal position of regression lines to be significant at the one per cent level of probability.

7. In *H. oregonensis,* weight-specific oxygen consumption is higher when acclimated to 25% sea water, at all acclimation temperatures than when acclimated to 75% and at the same series of temperatures. In the case of *H. nudus* the respiratory rate is higher when crabs are acclimated to the low temperature, low salinity combination, when compared with low temperature, high salinity. At higher acclimation temperatures, crabs acclimated to 75% sea water have the higher rate. Statistical analysis of ordinal position of regression lines for both species shows them to be significant at the one per cent level.

8. When *H. oregonensis* is acclimated to low salinity and any of the four acclimation temperatures, there is a differential size effect; weight-specific oxygen consumption of small crabs shows a proportionately greater change as acclimation temperature increases. And as acclimation temperature increases, size dependence decreases. Changes in slopes (b) for the total of the four acclimation temperature regression lines are significant at the one per cent level. If crabs are acclimated to the high salinity, no size effect is demonstrable. Comparison of the two acclimation salinities at each acclimation temperature also shows no size effect. For *H. nudus,* animals acclimated to low salinity and the four acclimation temperatures show no size effect, but at 75% sea water there is a demonstrable size effect. Weight-specific oxygen consumption of large animals shows a greater rate change at higher acclimation temperatures, and there is greater size dependence at these higher temperatures. Significance of the regression lines is at the five per cent level. No statistically significant differences in slope exist when the respiratory rate is compared for both salinities at each acclimation temperature.

9. Positive linear regression coefficients for these experimental results range from 0.315 to 0.667. Only a few approach the reported 0.67 exponent. These data show that the regression coefficient is not an inherent species character, but is dependent upon intrinsic and extrinsic factors.

10. Values of Q_{10} have been used to compare seasonal rate-temperature data, but show little consistency in relation to acutely measured responses as a function of increasing experimental temperature, size increase or seasonal difference. Simi-

larly, the temperature and salinity acclimation data fail to show a definite trend for size increase or for increase in temperature at a given size. Generally, Q_{10} values increase at lower acclimation temperatures, at both salinities, and then decrease as the temperatures increase.

11. Salinity affects the metabolic response of these two species of crabs to temperature. Weight-specific oxygen consumption is highest at the low temperature, low salinity combination. As temperature increases rate of oxygen consumption remains higher at the low salinity for *H. oregonensis*, but high salinity results in a higher rate for *H. nudus*. This greater response to low salinity is thought, in part, to be the result of increased work to maintain an osmotic balance. It does not result from increased muscular activity.

12. Differential responses of *Hemigrapsus* to temperature and salinity, as measured by oxygen consumption and temperature tolerance, are suggested as a means by which *H. nudus*, in particular, became established in the geographic area of this study.

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